

Deanna Garner
Editor

Phytosterols

Food Sources, Functions
and Health Benefits

PROGRESS IN FOOD SCIENCE AND TECHNOLOGY



Complimentary Contributor Copy

PROGRESS IN FOOD SCIENCE AND TECHNOLOGY

PHYTOSTEROLS
FOOD SOURCES, FUNCTIONS
AND HEALTH BENEFITS

No part of this digital document may be reproduced, stored in a retrieval system or transmitted in any form or by any means. The publisher has taken reasonable care in the preparation of this digital document, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained herein. This digital document is sold with the clear understanding that the publisher is not engaged in rendering legal, medical or any other professional services.

Complimentary Contributor Copy

PROGRESS IN FOOD SCIENCE AND TECHNOLOGY

Additional books in this series can be found on Nova's website
under the Series tab.

Additional e-books in this series can be found on Nova's website
under the e-book tab.

Complimentary Contributor Copy

PROGRESS IN FOOD SCIENCE AND TECHNOLOGY

PHYTOSTEROLS
FOOD SOURCES, FUNCTIONS
AND HEALTH BENEFITS

DEANNA GARNER
EDITOR



Complimentary Contributor Copy

Copyright © 2015 by Nova Science Publishers, Inc.

All rights reserved. No part of this book may be reproduced, stored in a retrieval system or transmitted in any form or by any means: electronic, electrostatic, magnetic, tape, mechanical photocopying, recording or otherwise without the written permission of the Publisher.

We have partnered with Copyright Clearance Center to make it easy for you to obtain permissions to reuse content from this publication. Simply navigate to this publication's page on Nova's website and locate the "Get Permission" button below the title description. This button is linked directly to the title's permission page on copyright.com. Alternatively, you can visit copyright.com and search by title, ISBN, or ISSN.

For further questions about using the service on copyright.com, please contact:

Copyright Clearance Center

Phone: +1-(978) 750-8400 Fax: +1-(978) 750-4470 E-mail: info@copyright.com.

NOTICE TO THE READER

The Publisher has taken reasonable care in the preparation of this book, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained in this book. The Publisher shall not be liable for any special, consequential, or exemplary damages resulting, in whole or in part, from the readers' use of, or reliance upon, this material. Any parts of this book based on government reports are so indicated and copyright is claimed for those parts to the extent applicable to compilations of such works.

Independent verification should be sought for any data, advice or recommendations contained in this book. In addition, no responsibility is assumed by the publisher for any injury and/or damage to persons or property arising from any methods, products, instructions, ideas or otherwise contained in this publication.

This publication is designed to provide accurate and authoritative information with regard to the subject matter covered herein. It is sold with the clear understanding that the Publisher is not engaged in rendering legal or any other professional services. If legal or any other expert assistance is required, the services of a competent person should be sought. FROM A DECLARATION OF PARTICIPANTS JOINTLY ADOPTED BY A COMMITTEE OF THE AMERICAN BAR ASSOCIATION AND A COMMITTEE OF PUBLISHERS.

Additional color graphics may be available in the e-book version of this book.

Library of Congress Cataloging-in-Publication Data

Library of Congress Control Number: 2015946403

ISBN: 978-1-63483-518-3 (eBook)

Published by Nova Science Publishers, Inc. † New York

Complimentary Contributor Copy

CONTENTS

Preface		vii
Chapter 1	Phytosterols in Nutraceuticals <i>Patrícia Vicente, Maria da Conceição Castilho and Fernando Ramos</i>	1
Chapter 2	Food Sources and Chemistry of Plant Sterols and Stanols <i>Raquel de Pinho Ferreira Guiné</i>	23
Chapter 3	Recovery of Phytosterols from Refining By-Products of Vegetable Oils <i>M. Victoria Ruiz-Méndez, Joaquín Velasco and Gloria Márquez-Ruiz</i>	43
Chapter 4	Phytosterols in Olive Oil: Overview of Analytical Methodology <i>Ibrahim M. Abu-Reidah and Amani Taamalli</i>	63
Chapter 5	Phytosterols: Applicability as Cholesterol Metabolism Biomarkers and Health Benefits <i>Isabel Andrade, Lélita Santos and Fernando Ramos</i>	85
Chapter 6	The Practical Use of Some Non-Cholesterol Sterols (Lathosterol, Desmosterol, Campesterol and Sitosterol) in Differentiation and Treatment of Children and Adolescents Suffering from Hypercholesterolemias <i>Josef Hyánek, František Pehal, Ladislava Dubska, Vera Martinikova, Jana Privarova and Ludek Taborsky</i>	97
Chapter 7	Phytosterols and Health Benefits: GC-MS Determination of Surrogate Markers for Cholesterol Synthesis/Absorption in Portuguese Young People <i>Rute Semedo, David Saraiva, Maria da Conceição Castilho, José Manuel Silva and Fernando Ramos</i>	119

Complimentary Contributor Copy

Chapter 8	Influence of Factors that Modulate Efficacy of Phytosterols <i>Milagros Rocha, Celia Bañuls, Susana Rovira-Llopis, Carlos Morillas, Silvia Veses, Sandra López-Domenech, Noelia Diaz-Morales, Victor Manuel Victor and Antonio Hernández-Mijares</i>	127
Chapter 9	Phytosterols Distribution in Alimentary Oil and Fingerprint <i>Kelly S. Bezerra and Nelson R. Antoniosi Filho</i>	163
Chapter 10	Estimation of Plant Sterol Intake in a Resident Population of the Valencian Community (Spain) <i>Guadalupe Garcia-Llatas, Evelyn Mejía, Antonio Cilla, Amparo Alegria and María Jesús Lagarda</i>	185
Index		207

Complimentary Contributor Copy

PREFACE

The consumption of phytosterols (plant sterols and/or stanols) can reduce cardiovascular risk by reducing hypercholesterolemia. In addition to reducing intestinal cholesterol absorption, phytosterols (PS), which are structurally similar to cholesterol, have other potential beneficial effects in humans. There are a wide range of plants that have phytosterols in their composition. In this book, the food sources of phytosterols are explored. The authors evaluate whether a decrease in carotenoids associated with the consumption of a therapeutic dose of PS alters oxidative stress parameters. Furthermore, the characterization of oils by phytosterols analysis was studied and is examined in this book. An overview of phytosterols in olive oil is given as well, particularly, the use of HPLC and GC coupled to various detection systems to isolate sterols from olive oil in order to focus on the occurrence of phytosterols and their role in human health.

Chapter 1 – Cardiovascular diseases are the primary cause of mortality and morbidity in the world and are associated with clearly higher levels of total cholesterol and low density lipoprotein. Phytosterols are cholesterol-like molecules found in all vegetal-derived foods items, but consumption of these compounds has a cardioprotective effect by lowering blood cholesterol levels. The efficacy, effectiveness and safety of phytosterols as a cholesterol-lowering therapy has already been corroborated in numerous clinical trials and meta-analyses by a dose of 2 g/day. However, the estimated average intake of naturally occurring plant sterols in the diet constitutes only about 10-20% of the recommended daily intake of 2 g and it is therefore necessary to ingest phytosterols-enriched foods or phytosterols supplements in order to reach the optimal dose.

Currently, various products enriched with phytosterols, including margarine, yogurt, milk, juice, cereal bars or nutraceuticals (tablets or capsules) are available on the market. With the increasing growth of this market it is important to alert for the risks inherent to the indiscriminate consumption of this type of products, and also to control the quality and their possible adulteration, in order to ensure a high level of protection of public health.

Qualitative and quantitative determination of phytosterols is well-documented in various food matrices as in vegetables, oil, fruit, cereal, bread, margarine and most recently, in milk and yoghurt. However for nutraceutical tablet or capsule forms there are no published methods. For this reason, it is essential to develop methodologies for the analysis of phytosterols in different food matrices, since each matrix is unique.

It seemed important to address the quality and the safety of phytosterol nutraceutical forms, contributing to the development of cholesterol-lowering products safety.

Complimentary Contributor Copy

Thus, in the present chapter, a GC-MS chromatographic methodology is developed, optimized and validated for the determination of phytosterols in nutraceuticals. The application of the referred methodology to a nutraceutical formulation, shows that five phytosterols were present, namely campesterol, campestanol, stigmasterol, β -sitostanol and β -sitosterol. The application of the developed methodology in quality control is also discussed.

Chapter 2 – A wide range of plants have phytosterols in their composition, both sterols and stanols, being however the stanols present in considerably smaller quantities when compared to sterols. Among the most abundant plant sterols are sitosterol, campesterol and stigmasterol. As to plant stanols, they are present in the diet in small amounts, being one of the most important the sitostanol.

Some food sources that are rich in phytosterols include for example: fruits, vegetables, nuts, seeds, cereals, legumes and vegetable oils. It is therefore important to know what the food sources of these bioactive compounds are and what amounts are present. This is one of the goals of the present chapter, which aims to revise the most recent scientific literature to look for updates regarding the food sources of phytosterols, as a complement to the information more generally available.

Both stanols and sterols are essential components of plant cell membranes and structurally resemble cholesterol. In fact, cholesterol is also a sterol, but, unlike plant sterols, it is predominantly of animal origin, being synthesized in the human liver. Because the bioactive effects of phytosterols are much associated to their chemical structure, also this aspect is addressed in the present chapter, by presenting the chemistry of the different molecules of the phytosterols.

Chapter 3 – Refining of oils allows the elimination of color, odor and flavor that make crude oils unacceptable to consumers. In addition, chemical compounds that might be toxic or those that decrease the oil stability are removed in the refining process. Unfortunately, other components including phytosterols, of well-known bioactivity, are removed in part along with the undesired compounds and are ultimately concentrated in the refining by-products. Therefore, re-use and exploitation of by-products as an attractive source of bioactive compounds is gaining much interest. In this chapter, by-products from both chemical and physical refining used as sources of phytosterols, namely soapstocks and deodorization distillates, will be discussed. Soapstocks consist mainly of sodium salts produced from free fatty acids and also contain neutral oil and gums. Deodorization distillates include a considerable number of bioactive compounds, among them phytosterols, that make them excellent starting materials for obtaining novel food products of high added value. The extraction, isolation and purification of phytosterols from these raw materials using diverse technologies, including molecular distillation, extraction with supercritical carbon dioxide, hydrolysis of esters and crystallization and re-crystallization of adducts, will be revised.

Chapter 4 – Recent interest in phytosterols stems from their potential protective role, through ingestion of plant foods and oils. This latter approach in turn, results in reducing of serum total cholesterol, and low density lipoprotein (LDL) which may delay or/and prevent occurrence of chronic diseases like CHD and stroke. Many plant foods and vegetable oils contain a variety of phytochemicals with several properties related to individual compounds such as phytosterols which are present in low quantities. For the isolation of phytosterols, high-performance liquid chromatography (HPLC) as well as gas chromatography (GC) coupled with either an ultraviolet/visible (UV/Vis), photodiode array (PDA) and a flame ionization detector (FID) in GC have been widely used. Moreover, mass spectrometry (MS)

Complimentary Contributor Copy

detector with different analyzers such as ion trap (IT), time-of-flight (Q) TOF, has been hyphenated for identification of phytosterols from diverse vegetable oils. In this sense, the use of HPLC and GC coupled to various detection systems to isolate sterols from olive oil has been reviewed in this chapter. This review is devoted to a short discussion of the occurrence of phytosterols, their role in human health, and focuses on a detailed presentation of the analytical methods, concluding with the advantages of analytical methods employed so far.

Chapter 5 – The consumption of phytosterols (plant sterols and/or stanols) can reduce cardiovascular risk, by reducing hypercholesterolemia. Serum cholesterol concentrations can be determined by the traditional lipid profile parameters (total cholesterol, LDL-C, HDL-C, triglyceride), and in addition, the cholesterol metabolism can also be studied by the quantification of the intestinal cholesterol absorption and endogenous cholesterol synthesis markers. The disruption of the cholesterol homeostasis between absorption and synthesis of cholesterol is related to a positive history of several cardiovascular diseases and will be focused in this chapter.

Sitosterol and campesterol are the major sources of phytosterols in diet, and are therefore the main biomarkers used to study the exogenous cholesterol absorption, while desmosterol and lathosterol, the late precursors of the cholesterol biosynthetic pathway, are the most common biomarkers used to quantify cholesterol synthesis, whether in diet/lifestyle interventions or drug therapy monitoring.

There is evidence that several food matrices enriched with phytosterols, or supplements, are effective in lowering serum LDL-C concentrations, when used alone as part of the diet or when used as an adjuvant to ongoing statin therapy. In addition, the market for functional foods and dietary supplements with a health claim (for lipid-lowering) is expanding rapidly worldwide, and consequently an increasing number of persons will use these products and combine them with their prescribed drugs. Therefore, several studies have investigated the effect on lipids and also on serum cholesterol metabolism markers of a variety of phytosterol-enriched food matrices in hypercholesterolemic patients and in statin-treated individuals.

In addition to the hypocholesterolemic effect of the phytosterol-enriched food products, it is considered that in the particular case of the fermented dairy (low-fat) products, there are other benefits, such as nutritional, physiological and immunological positive overall effects on health, and in particular, in intestinal health.

In sum, this chapter will include a description of phytosterol-enriched food matrices studied/used to lower cholesterol levels and developments and trends in the field will be discussed. Studies on the use of these functional foods in hypercholesterolemic patients, on and off drug therapy, will be referenced. The use of the cholesterol metabolism markers will be highlighted in regard to the study of cardiovascular diseases, and the potential role of phytosterols will be discussed in disease prevention and health promotion.

Chapter 6 – *Background*: The article presents selected plasmatic cholesterol precursors (Lathosterol – Lat, Desmosterol – Des) and plasmatic phytosterols (Campesterol—Cam, Sitosterol—Sit) in relation to the parameters of cholesterol metabolism in 300 children patients (8-18 yrs) with genetically proved heterozygous familial hypercholesterolemias (HFH) and other hypercholesterolemias in order to obtain practical experience with the possible differential and monitoring use of these parameters in dietary or drug treatment. The authors discuss their 20 years of experience with the use of these selected non-cholesterol sterols (NCS) in diagnosing, differentiation, and dietary & drug treatment.

Complimentary Contributor Copy

Methods: The plasma NCS values were determined using GC/MS; plasma lipid profile was established by routine clinical biochemistry methods; and molecular genetic analyses were conducted by PCR-SSCP and PCR-DGGE methods.

Results: The article indicates reference values of NCS for genetically proved healthy children of different ages. Patients with the so-called 'alimentary hypercholesterolemia' possess normal values of NCS. Children patients with HFH detected by positive selective screening (5th and 13th year of life) and arriving at the lipid outpatient clinic had usually been put on a low cholesterol diet (LCD) by pediatricians and had elevated levels of Lat and Des and compensatorily elevated Cam and Sit. Administration of simvastatin in sufficient therapeutic doses reduced Lat or Des ($p > 0.001$). Compensatory elevation of Cam and Sit persists in a few patients. Ezetimibe application decreases efficiently Cam and Sit rather than Lat and Des. Combined application of simvastatin with ezetimibe is the most efficient not only in the decrease in the plasma LDL-cholesterol, but also in decrease in Lat, Des as well as Cam and Sit. The diagnostic and therapeutic monitoring in patients with different types of hypercholesterolemia is also given in the graphic form.

Conclusions: Extending the laboratory monitoring profile by precursors of cholesterol synthesis (Lat, Des) and cholesterol absorption (Cam, Sit) improves the differential diagnosis power for different hypercholesterolemias in children and makes monitoring and treatment of children and adolescents suffering from HFH more precise and effective.

Chapter 7 – Early development of cardiovascular diseases is clearly associated with high plasma or serum cholesterol concentrations. Cholesterol homeostasis is reflected by the balance between cholesterol absorption vs. cholesterol synthesis. The ratios to serum cholesterol of sterol precursors reflect synthesis rate of cholesterol, while those of phytosterols reflect cholesterol absorption. A study with 95 volunteer healthy students from Coimbra University was developed in order to determine the prevalence of surrogate markers for cholesterol synthesis and absorption. Blood was collected and centrifuged and the serum was submitted to alkaline saponification, followed by *n*-hexane extraction and subsequently evaporation to dryness under a nitrogen stream. The dry residue was derivatized and the cholesterol and non-cholesterol sterols were determined by Gas Chromatography-Mass Spectrometry. Obtained results showed two relevant facts. Firstly, total cholesterol concentrations in the study population were quite high (21% of analyzed students with > 200 mg/dL), given the age group (19 to 24 years). Secondly, the cholesterol absorption markers (phytosterols) were found in concentrations higher than the markers of synthesis.

Obtained results can become an important contribution for developing better treatments to maintain healthy cholesterol concentrations, either for the elaboration of dietetic plans, namely for Mediterranean young people.

Chapter 8 – *Background:* Phytosterols (PS), which are structurally similar to cholesterol, reduce intestinal cholesterol absorption and have other potentially beneficial effects in humans. Based on recent meta-analyses, a PS intake of 2 g/day lowers LDLc by an average of 8-10%, though this effect varies depending on intrinsic and extrinsic factors particular to the individual. We have assessed whether baseline cholesterol concentration, amount of cholesterol in the diet, Apolipoprotein (Apo) E genotype, and presence of metabolic syndrome (MetS) influence the effectiveness of PS. Furthermore, the authors have evaluated whether a decrease in carotenoids associated with consumption of a therapeutic dose of PS alters oxidative stress parameters.

Complimentary Contributor Copy

Material and methods: This dietary intervention study was performed in moderate hypercholesterolemic and normocholesterolemic subjects. The study was of a parallel, randomized design with three arms, and was conducted over a period of 6 months. The first arm was performed with 52 volunteers from the general population who continued their usual diet plus 2 g PS/day administered in 500 ml of low fat milk for 3 months. In the other two arms, 75 hypercholesterolemic individuals were administered a healthy diet for 3 months in order to standardize dietary habits. After this adjustment, subjects were divided into two intervention groups: one group followed a healthy diet with 2 g of PS/day for 3 months, and the other group followed the healthy diet without PS enrichment. Anthropometric parameters, lipid profile, Apo E genotype, oxidative stress parameters (susceptibility to oxidation in LDL particles, total antioxidant capacity and lipid peroxidation) and concentrations of serum carotenoids and PS (β -sitosterol and campesterol) were measured at the beginning and end of the study.

Results: Three-month dietary supplementation with PS produced a significant decrease in blood cholesterol (5.3% in total cholesterol and 6.5% in LDLc) in the general population without dietary restrictions. The reduction of LDLc was more pronounced in subjects with higher baseline concentrations of cholesterol. The beneficial effect of PS on the lipid profile of mildly hypercholesterolemic patients was not affected by the amount of saturated fat or cholesterol in their diet, nor was it mediated by the genotype of the Apo E. PS therapy appeared to be of little value to MetS patients, probably because of the reduced intestinal absorption of cholesterol. On the other hand, consumption of PS did not reduce serum total antioxidant capacity or increase oxidative stress.

Conclusions: Our results demonstrate that the efficacy of PS does not depend on the composition of macronutrients in the diet or the apolipoprotein E genotype; on the contrary, it is subject to baseline levels of LDL cholesterol. Moreover, PS therapy appears to be of little value to MetS patients.

Chapter 9 – Phytosterols are lipid compounds present in eukaryotic cells of plant species. The intracellular synthesis of phytosterols part of a complex of reactions starting from terpenoid alcohols, thus forming the structure of squalene. The group of tetracyclic fused rings has a flexible side chain whose structural changes between sterols involve differences in this chain. The phytosterols include several different combinations of atoms resulting in different possible structures, which they were identified in several plant oils. The β -sitosterol is the main phytosterols, and we can not underestimating occurrence of stigmasterol and campesterol. Phytosterols may appear to genuinely and mainly as free and conjugated steroids (FS, ES, SG and ASG). However, the profile of steroids, or the types of steroids and their relative proportions are not equal, thus making their fingerprint of identification. These compounds have been highlighted because of the chemical and pharmaceutical applications, especially with medical-therapeutic purposes. Another function of phytosterols, and not least important, is their composition used in the characterization of oils and the fingerprint of the same. The characterization of oils by phytosterols analysis has been studied in order to detect the adulteration of noble oils. Various methods have been used in the isolation of steroids and subsequent identification and quantification of the same.

Chapter 10 – Plant sterols (PS), including phytosterols and phytostanols, are bioactive compounds which when consumed have several beneficial effects on the human body, including antiinflammatory and anticancer activities. In addition, a daily intake of 1.5-3 g PS results in an 8-10% reduction in LDL-cholesterol levels. Previous studies in northern

Complimentary Contributor Copy

European countries have reported PS intakes from natural and PS-enriched sources, though no recent studies have been made in the Mediterranean area, where the diet is known to be naturally rich in PS-containing foodstuffs (e.g., olive oil, nuts, etc.). Therefore, the aim of the present study was to describe the common PS sources and daily intakes of PS in a resident population (n = 75, 50.7% men, age = 20-68 years) in the Valencian Community (a region located on the Spanish Mediterranean coast) between November 2013 and May 2014, and to compare it with data collected in the year 2005 from a similar type of survey conducted among residents of the same region (n = 70, 47.1% men, age = 20-77 years). Intake was estimated by using a food frequency questionnaire considering naturally PS-containing food products from the normal diet. The results showed total PS intake to be similar in both study periods (274 mg/day in 2013-2014 versus 282 mg/day in 2005). Although the predominant PS content in natural sources corresponded to oils followed by nuts and legumes, on considering their frequency of consumption, the main PS sources were seen to be legumes > vegetables > oils. In sum, the recorded PS intake falls within the range reported by other studies in European populations (211-325 mg/day).

Complimentary Contributor Copy